# Effects of Different Pyrethroids on Landing Behavior of Female *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus* Mosquitoes (Diptera: Culicidae)

MIRIAM F. COOPERBAND[1] AND SANDRA A. ALLAN

Center for Medical, Agricultural and Veterinary Entomology, USDA–ARS, 1600 SW 23rd Drive, Gainesville, FL 32608

J. Med. Entomol. 46(2): 292–306 (2009)

**ABSTRACT** Mosquitoes from three genera, *Aedes aegypti* L., *Anopheles quadrimaculatus* Say, and *Culex quinquefasciatus* Say, were tested for facultative landing and resting behavior on pyrethroid-treated surfaces paired with adjacent untreated surfaces. The three pyrethroids tested were bifenthrin, deltamethrin, and lambda-cyhalothrin. Landing and resting behavior was video recorded and quantified using Observer XT software. Untreated control treatments were tested to show behavior in the absence of insecticides. In controls, the three species had different activity levels, with *An. quadrimaculatus* being the most active and *Cx. quinquefasciatus* being the least active. The three species had unique responses to different compounds tested. Landing frequency on adjacent untreated and treated filter papers did not differ for any compound or species at any time during the experiment. However, landing frequencies did differ between treatments and over time. Differences between treated and untreated sides were largely caused by changes in the length of time mosquitoes rested on each side. *An. quadrimaculatus* had a unique response to the presence of deltamethrin compared with the other species or compounds in which it spent an increased amount of time in contact with both treated and adjacent untreated surfaces. *Cx. quinquefasciatus* avoided all three compounds by the end of the experiment and rested longer on untreated sides. In most cases, modification of landing and resting behaviors occurred only after mosquitoes had the opportunity to come into contact and acquire a dose of pyrethroid. Bifenthrin had the fastest $TK_{50}$ for all species. Other differences between compounds for each species are described. The term excito-repellency has produced confusion in the literature, and it is revisited and discussed with respect to the results, which justify the use of alternative terminology. The term "locomotive stimulant" is offered as an acceptable alternative.

**KEY WORDS** insect behavior, contact irritant, sublethal effects of insecticide, excitation, barrier

With worldwide distribution, mosquitoes from the genera *Anopheles, Aedes,* and *Culex* are important vectors of many disease pathogens such as malaria, filariasis, dengue, yellow fever, Rift Valley fever, arboviral encephalitides, and other important diseases. Personal protection from mosquito bites and reduction of mosquito populations are among the subjects of research aimed to reduce disease transmission.

The use of insecticidal residues is one of the commonly used methods of chemical control. Insecticide-treated bed nets, curtains, military uniforms, barriers, and walls inside houses are some of the approaches for using residues to reduce human–vector contact and interrupt disease transmission (Roberts and Alecrim 1991, Perich et al. 1993, Eamsila et al. 1994, Roberts et al. 1997, Ansari et al. 1998, Takken 2002, Kapoor and Ansari 2003, Pates and Curtis 2005, Coleman and Hemingway 2007). These approaches commonly use synthetic pyrethroids, a class of neurotoxins that act on nerve axon sodium channels in a way similar to DDT (Vijverberg et al. 1982). Permethrin-treated bed nets and uniforms rely on the human host as the attractant or bait to lure mosquitoes into contact with the treated material long enough to deliver a lethal dose of insecticide (Miller and Gibson 1994). When treating resting sites such as the walls inside of houses, or a foliage barrier outside, there is no attraction involved, so contact with these treated surfaces is facultative and alternative untreated surfaces are available. Pyrethroids are commonly used to treat these surfaces because of their high activity and efficacy at low concentrations and their relative safety with regard to vertebrates and the environment (Corbel et al. 2004). However, pyrethroids, are known to produce "excito-repellency" (Evans 1993, Kongmee et al. 2004), or excitation resulting in movement away from the treated area, similar to responses observed with sublethal exposure to DDT (Kennedy 1947). When mosquitoes land on pyrethroid-treated surfaces in the absence of an attractant, excito-repellency may preclude

[1] Corresponding author: USDA–APHIS–PPQ–CPHST, Bldg. 1398, West Truck Road, Otis ANGB, MA 02542 (e-mail: Miriam.F.Cooperband@aphis.usda.gov).

| **Report Documentation Page** | | | *Form Approved*<br>*OMB No. 0704-0188* |
|---|---|---|---|
| Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. | | | |
| 1. REPORT DATE<br>**MAR 2009** | 2. REPORT TYPE | | 3. DATES COVERED<br>**00-00-2009 to 00-00-2009** |
| 4. TITLE AND SUBTITLE<br>**Effects of Different Pyrethroids on Landing Behavior of Female Aedes aegypti, Anopheles quadrimaculatus, and Culex quinquefasciatus Mosquitoes (Diptera: Culicidae)** | | | 5a. CONTRACT NUMBER<br>5b. GRANT NUMBER<br>5c. PROGRAM ELEMENT NUMBER |
| 6. AUTHOR(S) | | | 5d. PROJECT NUMBER<br>5e. TASK NUMBER<br>5f. WORK UNIT NUMBER |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br>**U.S. Department of Agriculture -ARS,Center for Medical, Agricultural and Veterinary Entomology,1600 SW 23rd Drive,Gainesville,FL,32608** | | | 8. PERFORMING ORGANIZATION REPORT NUMBER |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | | | 10. SPONSOR/MONITOR’S ACRONYM(S)<br>11. SPONSOR/MONITOR’S REPORT NUMBER(S) |
| 12. DISTRIBUTION/AVAILABILITY STATEMENT<br>**Approved for public release; distribution unlimited** | | | |
| 13. SUPPLEMENTARY NOTES | | | |

14. ABSTRACT

**Mosquitoes from three genera, Aedes aegypti L., Anopheles quadrimaculatus Say, and Culex quinquefasciatus Say, were tested for facultative landing and resting behavior on pyrethroidtreated surfaces paired with adjacent untreated surfaces. The three pyrethroids tested were bifenthrin deltamethrin, and lambda-cyhalothrin. Landing and resting behavior was video recorded and quanti?ed using Observer XT software. Untreated control treatments were tested to show behavior in the absence of insecticides. In controls, the three species had different activity levels, with An. quadrimaculatus being the most active and Cx. quinquefasciatus being the least active. The three species had unique responses to different compounds tested. Landing frequency on adjacent untreated and treated ?lter papers did not differ for any compound or species at any time during the experiment. However, landing frequencies did differ between treatments and over time. Differences between treated and untreated sides were largely caused by changes in the length of time mosquitoes rested on each side. An. quadrimaculatus had a unique response to the presence of deltamethrin compared with the other species or compounds in which it spent an increased amount of time in contact with both treated and adjacent untreated surfaces. Cx. quinquefasciatus avoided all three compounds by the end of the experiment and rested longer on untreated sides. In most cases, modi?cation of landing and resting behaviors occurred only after mosquitoes had the opportunity tocomeinto contact and acquire a dose of pyrethroid. Bifenthrin had the fastest TK50 for all species. Other differences between compounds for each species are described. The term excito-repellency has produced confusion in the literature, and it is revisited and discussed with respect to the results, which justify the use of alternative terminology. The term ?locomotive stimulant? is offered as an acceptable alternative.**

15. SUBJECT TERMS

<table>
<tr><td colspan="3">16. SECURITY CLASSIFICATION OF:</td><td rowspan="2">17. LIMITATION OF ABSTRACT<br>**Same as Report (SAR)**</td><td rowspan="2">18. NUMBER OF PAGES<br>**15**</td><td rowspan="2">19a. NAME OF RESPONSIBLE PERSON</td></tr>
<tr><td>a. REPORT<br>**unclassified**</td><td>b. ABSTRACT<br>**unclassified**</td><td>c. THIS PAGE<br>**unclassified**</td></tr>
</table>

them from staying in contact with the treated surface long enough to acquire a lethal dose. Furthermore, if excitation or repellency results from contact with pyrethroid vapors, mosquitoes may be less likely to land on treated surfaces in the absence of an attractant when alternative landing sites are available.

Our goal was to examine whether mosquitoes show a preference between treated and untreated surfaces when offered no incentive for landing on one over the other, to describe the sublethal effect of residual pyrethroids on the landing and resting behaviors of mosquitoes, and to determine whether differences exist between species and insecticides. Three representative species from each of the three major genera, *Aedes aegypti* L., *Anopheles quadrimaculatus* Say, and *Culex quinquefasciatus* Say, were selected for this study and tested with the pyrethroids bifenthrin, deltamethrin, and lambda-cyhalothrin.

## Materials and Methods

**Mosquitoes.** Mosquitoes were reared, as described by Gerberg et al. (1994), in the insectary at the Center for Medical, Agricultural and Veterinary Entomology, at the USDA in Gainesville, FL. Nonresistant strains of three species were used: *Ae. aegypti*, *An. quadrimaculatus*, and *Cx. quinquefasciatus.* Pupae in water cups were transferred to screened cages (30 by 30 by 30 cm) in an environmental chamber. They were allowed to emerge in a temperature-controlled chamber under a photoperiod of 16 L:8 D, at 22°C and 30% RH. Adult mosquitoes were provided a constant supply of 5% sugar-water solution and were allowed to mate in the cages. Female mosquitoes were used in bioassays ≈1 wk after adult eclosion. Behavioral bioassays were conducted using female mosquitoes during the daylight cycle in ambient laboratory temperature and relative humidity of 24–25°C and 18–20% RH.

**Bioassay.** Using the method described by Posey and Schreck (1981), 60 host-seeking female mosquitoes were lured toward the experimenter into a pint-sized paper food container (Neptune, Newark, NJ) with a screen bottom. They entered the paper container through a hole in the side (1 cm in diameter), which was later plugged with cotton. Through the same hole, an aspirator was inserted, and the 60 female mosquitoes were collected. At the start of the experiment, the mosquitoes were gently mouth aspirated from the aspirator into the bioassay chamber described below. Mosquitoes were tested in groups of 60 to ensure landing observations of less active species like *Cx. quinquefasciatus.* Latex gloves were worn to avoid contaminating surfaces with human skin odors.

A glass chamber (30.5 by 30.5 by 30.5 cm) was used to observe landing behavior on adjacent insecticide-treated and untreated filter papers attached to the inner back panel (Fig. 1). The front and back panels of the chamber were removable, and the entire apparatus was cleaned with acetone between treatments. The front glass panel contained a circular opening (8 cm in diameter) for access to the inside of the chamber. A plastic petri dish with a 2.5-cm-circular hole in the middle was taped over the opening of the front glass panel, and cotton was used to plug the hole in the petri dish during experiments. The small opening was used to insert the end of an aspirator for the introduction and removal of mosquitoes in the chamber, whereas the large opening was used after a trial to facilitate complete removal of all mosquitoes using a vacuum cleaner.

**Compounds and Sides.** Insecticides used were technical grade bifenthrin, deltamethrin, and lambda-cyhalothrin (Chem Service, West Chester, PA). Each pesticide was weighed and mixed with insecticide-grade acetone to make solutions that were serially diluted and applied to filter papers at the WHO recommended doses (Table 1) (Hougard et al. 2003). Each of these solutions was applied to soak a rectangular piece of filter paper (14.75 by 29.5 cm) in a glass jar. The paper was allowed to dry in the jar and was suspended in the air for ≈30 min. Disposable pipette tips were used, and the pipetter was cleaned with acetone between each treatment. The untreated (acetone alone) and treated (acetone with insecticide) filter papers were taped side-by-side on the removable rear glass panel of the bioassay chamber using clear adhesive tape at the corners. The panel was rotated 180° between replicates to alternate the sides of the treated and untreated papers in the chamber. A control was tested in which both papers were treated only with acetone.

**Video Recording and Analysis.** The bioassay chamber was illuminated by two infrared LED light sources located outside of the chamber (940 nm with arrays of 42 LEDs; Rainbow IRLC394; Rainbow CCTV, Costa Mesa, CA). Red acetate was placed over the top of the glass chamber to improve the video image (Roscolux Medium Red #27; Rosco Laboratories, Stamford, CT). The entire setup was surrounded by a white curtain held in place by a metal frame. The white curtain allowed diffuse ambient fluorescent light through but shielded the bioassay from other visual cues in the laboratory. The light level inside the bioassay chamber was 512 lux. An opening in the curtain near the top front provided a space to aim the video camera (Panasonic WV-BP334 with Rainbow lens 1/3" manual IR-sensitive, 3–8 mm, F1.4). The video was recorded using a Canopus Digital Video Recorder (EMR100) and MediaCruise software (version 2.24.001) and observed on a laptop computer, which faced away from the bioassay. For each replicate, 60 female mosquitoes were placed in the chamber, and their behavior was recorded for 30 min.

Videos were analyzed using The Observer XT (v. 6.1.40; Noldus Information Technology, Wageningen, The Netherlands). Using this software, every time a mosquito landed, the landing was designated an identifying number. The number it was designated depended on which side the mosquito landed (untreated or treated). Acetate film was taped to the computer monitor so that identification numbers could be written next to each mosquito with a water-soluble, felt-tipped pen. When a mosquito landed on a filter paper, its number was entered into The Observer, which

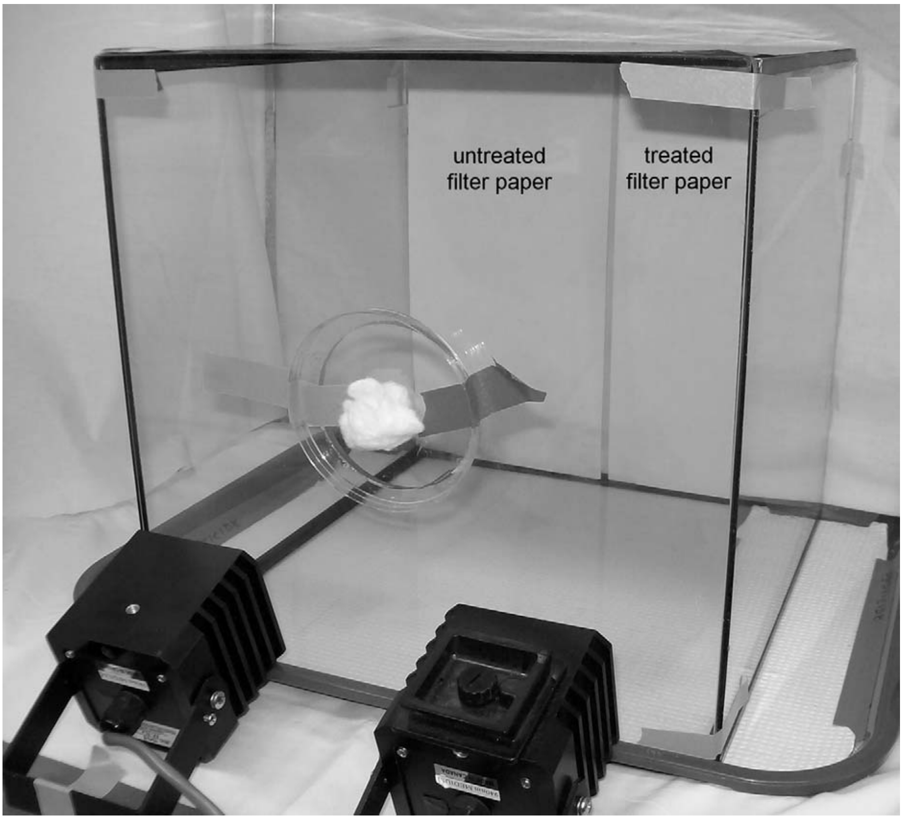


**Fig. 1.** The bioassay chamber, showing the two filter papers side-by-side on the back panel, the cotton-plugged opening, and the two IR lights.

recorded the start time. When the mosquito left the paper, its number was again entered into The Observer, which recorded the end time. At the end of each trial, data were checked by The Observer for errors and corrected by hand and exported into Microsoft Excel (Microsoft Office 2003 SP2). Each recording in Excel was placed in its own tab, and data were sorted and processed to determine the time each landing event started, the duration of each landing event, and the side (untreated or treated) each landing event took place.

**Landing Duration, Frequency, and Contact Time.** For each 30-min recording, the mean landing duration was calculated, as well as the number of landings, and the sum of all the time 60 mosquitoes cumulatively spent in contact with each filter paper over 30 min. Number of landings and sum of contact time were each divided by 60 to determine the mean number of landings and contact time per mosquito for each side.

**Snapshots over Time.** Snapshots of the number of mosquitoes resting on each piece of filter paper were quantified at 2.5, 5, 10, 15, 20, 25, and 30 min, and untreated and treated sides were compared with each other for each treatment. For each treatment, the change in number of mosquitoes resting on each filter paper at 2.5 min compared with the number resting at 30 min was also computed.

**Change in Landing Frequency and Duration over Time.** To determine whether mosquitoes modified their landing behavior after gaining exposure to insecticide, for each treatment and side, the landing frequency in each 5-min time increment was quantified and compared. Another test to determine whether mosquitoes modified their landing behavior

**Table 1. Application rates of insecticides and controls applied to filter papers (29.5 by 14.75 cm)**

| Insecticide | WHO recommended application rate ($mg/m^2$) | Actual application rate ($mg/m^2$) | AI (mg/ml acetone) | Solution applied to treatment (ml) | Acetone applied to untreated side (ml) | Purity of technical grade (%) |
|---|---|---|---|---|---|---|
| Bifenthrin | 25 | 25 | 0.157 | 7.036 | 7.036 | 99 |
| Deltamethrin | 25 | 20 | 0.157 | 5.629 | 5.629 | 98 |
| Lambda-cyhalothrin | 20 | 20 | 0.157 | 5.629 | 5.629 | 98 |

WHO-recommended application rates listed are according to Hougard et al. (2003).

after gaining exposure to insecticide looked at change in landing duration over time. Landing durations were averaged for two 15-min time increments and compared. For each treatment and side, the mean duration of landings from the first half of the trial were compared with those in the last half of the trial. To compare the change in behavior over time between treatments, rates of change in each replicate were computed and compared. Rate of change was computed as

$$\frac{(b_{t_2} - b_{t_1})}{(t_2 - t_1)}$$

where, in each replicate, $b$ was the average measured behavior over the given period of time, starting in time $t_1$ and ending in time $t_2$. Differences between treatments in landing frequency and rates of change of landing duration were compared for each species. Pairwise comparisons were made between sides for rates of change, as well as between early and late landing durations and frequencies on each side. The number of mosquitoes that landed at any time during the first 20 min and remained on the same surface until the end of the experiment was also quantified for each treatment and side.

**Ratio Between Sides.** To study the relationship between landing durations on adjacent untreated and treated sides over time, average landing durations on untreated sides were divided by average landing durations on treated sides over three 10-min increments and plotted as ratios.

**Knockdown.** A separate experiment was run to determine the time it took for 50% of the mosquitoes to be knocked down ($TK_{50}$) for each treatment. Treatments in the glass chamber were set up as described above except without video recording. Sixty female mosquitoes were aspirated into the box and checked periodically to count the number of mosquitoes that were knocked down. When the number of knocked down mosquitoes reached the mid-20s, they were observed continuously until 30 mosquitoes were knocked down, and the time was recorded. A mosquito was considered knocked down when it was lying on its side or back on the floor of the box, with none of its tarsi in contact with the floor. In some cases, $TK_{50}$ was far from being reached by 10 h, at which time they were not checked again until 24 h. For the few $TK_{50}$ observations that lasted >10 but <24 h, they were conservatively assigned a $TK_{50}$ of 10 h for the sake of the analysis. $TK_{50}$ was replicated five times for each treatment.

**Statistical Design and Analysis.** This experiment consisted of a 3 by 4 factorial design with factors being species (*Ae. aegypti*, *An. quadrimaculatus*, and *Cx. quinquefasciatus*) and compound (control, bifenthrin, deltamethrin, and lambda-cyhalothrin). Therefore, the experiment had 12 treatments, each of which had two filter papers side-by-side (untreated and treated) that were tested as repeated measures, and each trial was replicated five times. All statistical analyses were conducted using SAS for Windows (SAS Institute 2003).

**Table 2. Summary of activity increase (+) or decrease (−) with the presence of pyrethroids compared with controls for each species**

| | *Ae. aeg.* | | | *An. quad.* | | | *Cx. quinq.* | | |
|---|---|---|---|---|---|---|---|---|---|
| | bf | dm | lc | bf | dm | lc | bf | dm | lc |
| Untreated sides | | | | | | | | | |
| Mean bout duration (30 min) | | | | | | | − | | − |
| Mean contact time (30 min) | | | | | + | | | | |
| No. resting early versus late | | | | | | | + | | |
| Total no. resting until end | | + | | | ++ | + | | | − |
| Rate of change in landing duration over time | | | | | | | | | |
| Treated sides | | | | | | | | | |
| Mean bout duration (30 min) | − | | | | | | − | − | − |
| Mean contact time (30 min) | | | | | | | | − | |
| No. resting early versus late | | | | | | | − | − | − |
| Total no. resting until end | − | − | − | | + | | −− | −− | −− |
| Rate of change in landing duration over time | | | | | | | + | + | + |
| Both sides | | | | | | | | | |
| Landing frequency over time | + | − | | + | + | + | | | + |
| Mean landing frequency (30 min) | | | | | | | | | |
| Total contact time (sum of sides) | | | | | + | | − | − | − |

Doubled symbols indicate a marked change to or from zero. Blank spaces indicate there was no significant difference from the control. bf, bifenthrin; dm, deltamethrin; lc, lambda-cyhalothrin.

Data for each treatment and side were tested for normal distribution and homoscedasticity. Repeated-measures analysis of variance (ANOVA) was conducted on untreated sides and on treated sides to compare the treatments and their interactions on their respective sides (Montgomery 2005). Also, total contact time on untreated and treated sides were combined to compare the combined total contact time spent by each species in the presence of each compound. The Tukey test was conducted to separate means between combinations of factors when there were significant differences. For each treatment, paired Student's *t*-tests ($P < 0.05$) were used to find differences between the untreated and treated sides (Sokal and Rohlf 1995). Data from some treatments were not normally distributed, so mean total contact time and mean number landings were log-transformed for analyses (Sokal and Rohlf 1995). Untransformed data are reported here.

The number of mosquitoes on the treated side was compared with the untreated side at different times using pairwise comparisons. The paired Student's *t*-

**Table 3. (a) Means of average landing bout duration, average cumulative contact time per mosquito (untransformed), average no. of landings per mosquito (untransformed), and average rates of change of landing duration, on untreated (unt) and treated (trt) sides, accumulated when 60 mosquitoes were observed for 30 min per replicate; (b) means and significant differences transposed from (a) to highlight differences between species**

| Treatment | Mean landing duration (min)[a,b] | | | Mean total contact time per mosquito (min)[a,b] | | | Mean number landings per mosquito[a,c] | | Mean rate of change of landing duration (s/min)[b,d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | unt | trt | *N* | unt | trt | *N* | Per side | *N* | unt | trt | *N* |
| (a) Compound by species | | | | | | | | | | | |
| *Ae. aegypti* | | | | | | | | | | | |
| Control | 3.9a | 3.9abc | 5 | 2.1a | 2.5abcd | 5 | 0.7cde | 10 | 2.3a | −4.1ab | 5 |
| Bifenthrin | 1.8a | 1.0d | 5 | 2.2ab | 1.9bcde | 5 | 1.1bc | 10 | −9.5a* | −2.2ab | 5 |
| Deltamethrin | 4.8a* | 1.7bcd | 5 | 3.1ab* | 1.0de | 5 | 0.6cde | 10 | −15.5a* | −1.7ab | 5 |
| Lambda-cyhalothrin | 3.5a* | 1.7bcd | 5 | 2.6ab* | 1.2cde | 5 | 0.7cd | 10 | −9.5a* | −0.8a | 5 |
| *An. quadrimaculatus* | | | | | | | | | | | |
| Control | 1.2a | 1.6cd | 5 | 2.6ab | 2.9abcd | 5 | 2.0ab | 10 | −9.1a | −5.7ab | 5 |
| Bifenthrin | 1.1a* | 0.8d | 5 | 4.9bc* | 3.4abc | 5 | 4.6ab | 10 | −4.5a* | −2.1ab | 5 |
| Deltamethrin | 2.0a | 1.1d | 5 | 9.2c | 5.8a | 5 | 5.2a | 10 | −3.3a | −0.1a | 5 |
| Lambda-cyhalothrin | 1.6a | 1.2d | 5 | 4.6abc | 3.6ab | 5 | 3.1ab | 10 | −2.7a | −1.2a | 5 |
| *Cx. quinquefasciatus* | | | | | | | | | | | |
| Control | 15.3b | 15.0e | 5 | 4.2ab | 4.1abc | 5 | 0.3ef | 10 | −57.8b | −57.1c | 3 |
| Bifenthrin | 7.6a | 4.9a | 5 | 2.7ab | 1.9bcde | 5 | 0.5def | 10 | −53.7b* | −14.6b | 4 |
| Deltamethrin | 18.3b* | 4.1ab | 5 | 3.0ab* | 0.6e | 5 | 0.2f | 10 | −54.8b** | −4.9ab | 4 |
| Lambda-cyhalothrin | 5.8a | 3.0abcd | 5 | 2.2ab | 1.9bcde | 5 | 0.7de | 10 | −41.7b* | −13.3ab | 5 |
| ANOVA *F* | 16.61 | 54.75 | | 6.36 | 7.53 | | 29.17 | | 24.53 | 23.89 | |
| (b) Species by compound | | | | | | | | | | | |
| Control | | | | | | | | | | | |
| *Ae. aegypti* | 3.9a | 3.9abc | 5 | 2.1a | 2.5abcd | 5 | 0.7cde | 10 | 2.3a | −4.1ab | 5 |
| *An. quadrimaculatus* | 1.2a | 1.6cd | 5 | 2.6ab | 2.9abcd | 5 | 2.0ab | 10 | −9.1a | −5.7ab | 5 |
| *Cx. quinquefasciatus* | 15.3b | 15.0e | 5 | 4.2ab | 4.1abc | 5 | 0.3ef | 10 | −57.8b | −57.1c | 3 |
| Bifenthrin | | | | | | | | | | | |
| *Ae. aegypti* | 1.8a | 1.0d | 5 | 2.2ab | 1.9bcde | 5 | 1.1bc | 10 | −9.5a* | −2.2ab | 5 |
| *An. quadrimaculatus* | 1.1a* | 0.8d | 5 | 4.9bc* | 3.4abc | 5 | 4.6ab | 10 | −4.5a* | −2.1ab | 5 |
| *Cx. quinquefasciatus* | 7.6a | 4.9a | 5 | 2.7ab | 1.9bcde | 5 | 0.5def | 10 | −53.7b* | −14.6b | 4 |
| Deltamethrin | | | | | | | | | | | |
| *Ae. aegypti* | 4.8a* | 1.7bcd | 5 | 3.1ab* | 1.0de | 5 | 0.6cde | 10 | −15.5a* | −1.7ab | 5 |
| *An. quadrimaculatus* | 2.0a | 1.1d | 5 | 9.2c | 5.8a | 5 | 5.2a | 10 | −3.3a | −0.1a | 5 |
| *Cx. quinquefasciatus* | 18.3b* | 4.1ab | 5 | 3.0ab* | 0.6e | 5 | 0.2f | 10 | −54.8b** | −4.9ab | 4 |
| Lambda-cyhalothrin | | | | | | | | | | | |
| *Ae. aegypti* | 3.5a* | 1.7bcd | 5 | 2.6ab* | 1.2cde | 5 | 0.7cd | 10 | −9.5a* | −0.8a | 5 |
| *An. quadrimaculatus* | 1.6a | 1.2d | 5 | 4.6abc | 3.6ab | 5 | 3.1ab | 10 | −2.7a | −1.2a | 5 |
| *Cx. quinquefasciatus* | 5.8a | 3.0abcd | 5 | 2.2ab | 1.9bcde | 5 | 0.7de | 10 | −41.7b* | −13.3ab | 5 |
| ANOVA *F* | 16.61 | 54.75 | | 6.36 | 7.53 | | 29.17 | | 24.53 | 23.89 | |

Means in the same column, followed by the same letter are not significantly different from each other (Tukey means separation, $P > 0.05$). Rate of change of landing duration was calculated for each replicate using the equation defined in the methods. Note that the greater rates of change over time on the untreated sides reflect that landing durations were immediately shorter on treated sides, and only became shorter on untreated sides after some time.

[a] Means taken over the entire 30−min exposure.

[b] Repeated-measures ANOVA ($P = 0.05$).

[c] Mean no. of landings on untreated sides did not differ from treated sides, so sides were combined for the analysis (ANOVA, $P = 0.05$).

[d] For each replicate, mean landing durations in the last 15 min were subtracted from those in the first 15 min of each trial; zero indicates no change.

Significant difference between untreated and treated sides in paired Student's *t*-test with $P < 0.05$ (*) or $P < 0.001$ (**).

test ($P < 0.05$) was used for normally distributed data (Sokal and Rohlf 1995). However, some comparisons involved non-normally distributed data that were not rectified by log transformation, so in those cases, the Wilcoxon matched pairs signed rank test was used ($P < 0.05$) (Conover 1980). Treatments in the $TK_{50}$ study were compared using ANOVA and Tukey means separation ($P = 0.05$).

## Results

**Treatment Differences in 30 min.** A summary of significant differences between pesticide treatments and the control for each species is presented in Table 2. Means separations for landing duration, total contact time per mosquito, and number of landings per mosquito on untreated and treated sides of each treatment are presented in Tables 3a (sorted by species), 3b (sorted by compound), and Fig. 2. Two- and three-way interactions were found between species, compound, and side for landing duration and total contact time (Table 4; repeated-measures ANOVA, $P = 0.05$). Means separations showed that behaviors in controls differed by species, suggesting innate differences in the landing and resting behaviors of the three species tested (Table 3b). Repeated-measures ANOVA on

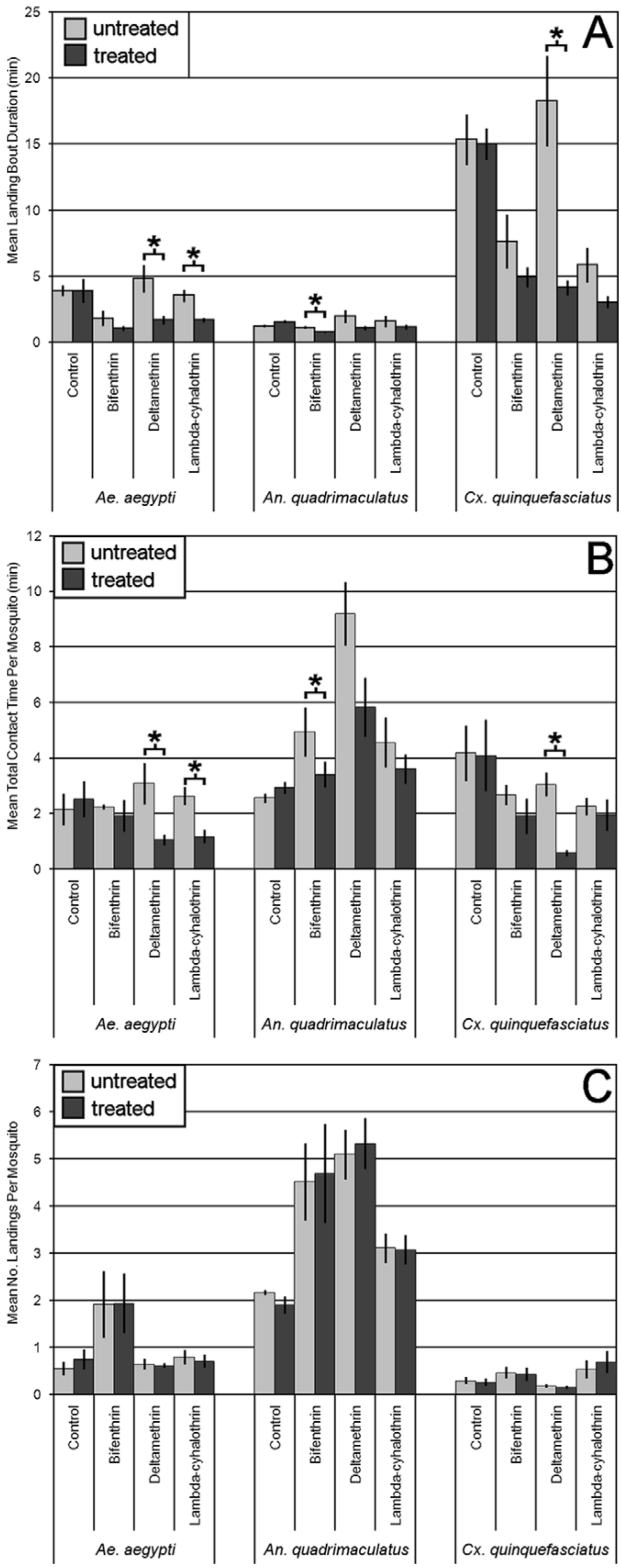


**Fig. 2.** Average landing duration (A), total contact time (B), and number of landings per mosquito (C) for untreated (light) and treated (dark) sides ($N = 5$). Asterisks indicate the untreated side was significantly different from the treated side (Student's paired $t$-test, $P < 0.05$). Bars represent SE. Note differences in scale between species.

**Table 4. ANOVA tables for average landing duration, total contact time, no. of landings, and rate of change, showing significant effects and interactions**

| Effect | df | Landing duration[a,b] | | Total contact time[a,b] | | No. landings[a,c] | | Rate of change of landing duration[b,d] | |
|---|---|---|---|---|---|---|---|---|---|
| | | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Species | 2 | 108.02 | <0.001 | 33.03 | <0.001 | 135.13 | <0.001[e] | 146.51 | <0.001 |
| Compound | 3 | 17.29 | <0.001 | 0.50 | 0.684 | 6.70 | <0.001 | 6.51 | 0.001 |
| Species × compound | 6 | 10.00 | <0.001 | 6.66 | <0.001 | 3.81 | 0.004 | 6.73 | <0.001 |
| Side | 1 | 34.86 | <0.001 | 35.72 | <0.001 | 0.16 | 0.689 | 104.32 | <0.001 |
| Side × species | 2 | 13.71 | <0.001 | 2.57 | 0.087 | 0.34 | 0.713 | 43.11 | <0.001 |
| Side × compound | 3 | 11.92 | <0.001 | 8.51 | <0.001 | 0.22 | 0.884 | 14.54 | <0.001 |
| Side × species × compound | 6 | 5.64 | <0.001 | 1.59 | 0.170 | 1.20 | 0.324 | 5.59 | <0.001 |

[a] Means taken over the entire 30-min exposure.
[b] Repeated-measures ANOVA ($P = 0.05$).
[c] Mean no. of landings on untreated sides did not differ from treated sides, so sides were combined for the analysis (ANOVA, $P = 0.05$).
[d] For each replicate, mean landing durations from the first 15 min were compared with those from the last 15 min of the exposure; zero indicates no change.
[e] This effect became nonsignificant after side was removed from the model ($P > 0.05$).

number of landings showed interactions between species and compound, but no significant effects were found for side (Table 4). Therefore, sides were combined to explore differences between treatments (ANOVA, $P = 0.05$).

Because there were many interactions, few strong patterns were immediately obvious. A general pattern appeared between species for landing duration and frequency, where *An. quadrimaculatus* usually landed most frequently, followed by *Ae. aegypti,* and then by *Cx. quinquefasciatus* with the lowest landing frequency (Table 3b). *Cx. quinquefasciatus* had, on average, less than one landing per mosquito on either side during the 30-min trials (Fig. 2c). Landing durations were usually longest for *Cx. quinquefasciatus,* followed by *Ae. aegypti,* and then by *An. quadrimaculatus* with the shortest landing durations. This pattern is apparent for each compound tested, but especially clear for the controls. However, the pattern did not occur in overall contact time over 30 min.

When total contact time on untreated and treated sides were combined, there were no differences between compounds for *Ae. aegypti,* and it was found that *An. quadrimaculatus* spent significantly more time in contact with surfaces when in the presence of deltamethrin than with the other three compounds (ANOVA, $P < 0.001$). For *Cx. quinquefasciatus,* when sides were combined, it was found that this species spent significantly more time in contact with surfaces in the control treatment than in the presence of any of the pyrethroids ($P = 0.035$; Table 2).

**Differences Between Sides in 30 min.** No differences were found between the two sides for any of the controls, indicating no bias between sides in the experimental apparatus. Pairwise comparisons between treated and untreated sides showed that each species responded differently to different compounds (Tables 3a, b; Fig. 2, see asterisks). *Ae. aegypti* females spent significantly less time in contact with sides treated with deltamethrin or lambda-cyhalothrin than their respective untreated sides. Conversely, *An. quadrimaculatus* females spent significantly less time on the bifenthrin-treated side than the untreated side. *Cx. quinquefasciatus* females spent significantly less time on the deltamethrin-treated side than the untreated side over 30 min. Treatments with significant differences between untreated versus treated sides for total contact time over 30 min also had significant differences between untreated versus treated sides for mean landing duration over 30 min. The average number of landings did not differ between untreated and treated sides for any of the treatments (Table 4).

**Snapshots of Mosquitoes over Time.** Pairwise comparisons were conducted to explore differences between the number of mosquitoes resting on untreated and treated sides at seven time increments up to 30 min (Table 5, see asterisks). No differences were found between the two sides of the controls for any of the species, indicating no directional bias in the experimental apparatus. All significant differences showed fewer mosquitoes resting on the treated sides. For *Ae. aegypti,* significantly fewer mosquitoes were on the treated sides for bifenthrin (between 15 and 30 min) and deltamethrin (from 5 to 20 min). For *An. quadrimaculatus,* no pattern of differences over time was evident for any of the compounds. For *Cx. quinquefasciatus,* all three compounds produced patterns of significant differences, starting from 10 or 15 min and continuing to 25 or 30 min.

When comparing the change, between 2.5 and 30 min, in the number of mosquitoes resting on each side, there were significant changes over time for certain treatments and sides (Table 5, see arrows). Under control conditions, there were no significant changes for any of the three species. In the presence of insecticides, each species responded differently to different compounds. For instance, with bifenthrin, there was a significant decrease in numbers of *An. quadrimaculatus* on both sides, whereas *Ae. aegypti* numbers only decreased on treated sides, and *Cx. quinquefasciatus* numbers decreased on treated sides but increased on untreated sides. With deltamethrin, there was a significant decrease over time in the number of *Ae. aegypti* mosquitoes resting on both surfaces, whereas *An. quadrimaculatus* numbers did not change over time, and *Cx. quinquefasciatus* numbers decreased only on

**Table 5. Average numbers of mosquitoes on untreated (unt) and adjacent treated (trt) surfaces in snapshots at different times ($N = 5$)**

| Species | Time (min) | Mean no. mosquitoes resting on filter paper | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | | | Bifenthrin | | | Deltamethrin | | | Lambda-cyhalothrin | | |
| | | unt | trt | (total) | unt | trt | (total) | unt | trt | (total) | unt | trt | (Total) |
| *Aedes aegypti* | | | | | | ↓ | | ↓ | ↓ | | | | |
| | 2.5 | 7.0 | 6.4 | (13.4) | 7.2 | 4.4 | (11.6) | 7.4 | 6.0 | (13.4) | 6.6 | 3.4 | (10.0) |
| | 5 | 5.8 | 4.8 | (10.6) | 7.4 | 1.8 | (9.2) | 7.4[a] | 2.4 | (9.8) | 5.0 | 3.0 | (8.0) |
| | 10 | 3.6 | 4.6 | (8.2) | 5.0 | 3.8 | (8.8) | 6.8[a] | 2.4 | (9.2) | 3.8 | 2.2 | (6.0) |
| | 15 | 3.0 | 3.6 | (6.6) | 6.0[a] | 2.6 | (8.6) | 5.8[a] | 1.2 | (7.0) | 4.4 | 2.2 | (6.6) |
| | 20 | 3.8 | 6.0 | (9.8) | 5.2 | 1.6 | (6.8) | 6.2[a] | 1.2 | (7.4) | 4.2 | 2.6 | (6.8) |
| | 25 | 3.6 | 5.8 | (9.4) | 5.4[a] | 2.0 | (7.4) | 4.8 | 2.2 | (7.0) | 5.6 | 1.6 | (7.2) |
| | 30 | 3.8 | 3.0 | (6.8) | 5.0[a] | 1.0 | (6.0) | 2.6 | 1.6 | (4.2) | 4.8 | 2.8 | (7.6) |
| *Anopheles quadrimaculatus* | | | | | ↓ | ↓ | | | | | | | |
| | 2.5 | 7.4 | 8.2 | (15.6) | 12.4 | 9.8 | (22.2) | 14.8 | 15.8 | (30.6) | 8.4 | 8.4 | (16.8) |
| | 5 | 5.0 | 5.8 | (10.8) | 12.4[a] | 5.0 | (17.4) | 15.8 | 12.8 | (28.6) | 7.4 | 5.2 | (12.6) |
| | 10 | 4.4 | 5.0 | (9.4) | 12.6 | 7.2 | (19.8) | 19.4 | 10.0 | (29.4) | 9.4 | 7.6 | (17.0) |
| | 15 | 3.6 | 4.6 | (8.2) | 9.6 | 7.6 | (17.2) | 18.2 | 12.4 | (30.6) | 10.0[a] | 6.6 | (16.6) |
| | 20 | 3.2 | 5.4 | (8.6) | 8.6 | 6.4 | (15.0) | 17.8 | 13.4 | (31.2) | 10.4 | 7.2 | (17.6) |
| | 25 | 5.4 | 6.4 | (11.8) | 8.2 | 5.6 | (13.8) | 16.8 | 12.8 | (29.6) | 9.0 | 7.2 | (16.2) |
| | 30 | 7.4 | 9.8 | (17.2) | 5.4 | 3.4 | (8.8) | 17.8 | 16.4 | (34.2) | 9.2 | 8.4 | (17.6) |
| *Culex quinquefasciatus* | | | | | ↑ | ↓ | | | ↓ | | | ↓ | |
| | 2.5 | 9.0 | 6.8 | (15.8) | 4.4 | 4.6 | (9.0) | 6.0 | 3.8 | (9.8) | 5.8 | 6.8 | (12.6) |
| | 5 | 9.2 | 7.4 | (16.6) | 5.4 | 4.4 | (9.8) | 6.0 | 3.8 | (9.8) | 5.8 | 7.4 | (13.2) |
| | 10 | 8.8 | 7.8 | (16.6) | 6.2[a] | 3.8 | (10.0) | 6.0 | 0.4 | (6.4) | 6.0 | 4.2 | (10.2) |
| | 15 | 8.6 | 8.0 | (16.6) | 6.4[a] | 2.4 | (8.8) | 6.6[a] | 0.4 | (7.0) | 7.2[a] | 2.0 | (9.2) |
| | 20 | 8.0 | 8.8 | (16.8) | 7.6[a] | 0.8 | (8.4) | 6.2[a] | 0.6 | (6.8) | 7.0[a] | 1.0 | (8.0) |
| | 25 | 7.6 | 9.2 | (16.8) | 7.6[a] | 1.4 | (9.0) | 6.2[a] | 0.2 | (6.4) | 6.4[a] | 1.0 | (7.4) |
| | 30 | 7.2 | 9.4 | (16.6) | 8.0[a] | 1.0 | (9.0) | 6.0[a] | 0.0 | (6.0) | 4.2 | 1.4 | (5.6) |

Numbers in parentheses represent the total sum of both sides. Each replicate contained 60 female mosquitoes.

[a] Significant difference between sides, paired Student's *t*-test ($P < 0.05$).

↓, significant decrease in no. of mosquitoes resting on a surface at 2.5 versus 30 min, paired Student's *t*-test ($P < 0.05$); ↑, significant increase in no. of mosquitoes resting on a surface at 2.5 versus 30 min, paired Student's *t*-test ($P < 0.05$).

the treated sides. With lambda-cyhalothrin, only *Cx. quinquefasciatus* showed a significant reduction over time on treated sides. Interestingly, the number of *An. quadrimaculatus* mosquitoes on either side in the deltamethrin treatment was approximately twice that of the control (Table 5).

**Landing Duration Change over Time.** When comparing landing durations that initiated early in the trial (between 0 and 15 min) to those that started late in the trial (between 15 and 30 min), there were significant differences between treated and untreated sides and between compounds and species (Tables 3a, b and 4; Fig. 3). Rate of change in landing durations from early to late in the trial were calculated and compared to show differences in change over time between treatments. For controls, change between the two sides did not differ significantly (Table 3a, b). Under control conditions, *Ae. aegypti* landing durations did not significantly change from early to late in the experiment, whereas both *An. quadrimaculatus* and *Cx. quinquefasciatus* reduced the durations of their landing bouts as the experiment progressed (Fig. 3, see asterisks). A pattern appeared where average rates of change were often closer to zero on pesticide-treated sides and generally decreased more sharply on adjacent untreated sides. A possible explanation for this pattern might be that mosquitoes that landed on the treated sides may have taken flight more quickly after exposure to insecticide, regardless of landing early or late in the experiment, resulting in no change over time. With time, the likelihood a mosquito had already come in contact with the treated side increased as the likelihood of unexposed mosquitoes landing on the untreated side decreased, possibly resulting in the greater rate of change seen on the untreated sides (Fig. 3).

The average numbers of mosquitoes that landed in the first 20 min and remained until the end of the experiment, and the average start times of those landings, were quantified for descriptive purposes (Table 6). This approach showed that the three species behaved differently under control conditions. In the controls, *Cx. quinquefasciatus* had the most, followed by *Ae. aegypti*, and *An. quadrimaculatus* had no mosquitoes that did this. In the presence of insecticides, essentially no *Cx. quinquefasciatus* or *Ae. aegypti* remained on treated sides, whereas more *An. quadrimaculatus* remained on both sides in the presence of deltamethrin (Table 6).

**Landing Frequency Change over Time.** Time played a significant role on landing frequency, and three-way interactions were found between time, species, and compound (ANOVA, $P < 0.05$). Separate ANOVA tests were run on each species for each time increment to determine differences in landing frequency between compounds (Table 7; Fig. 4). Differences in landing frequency were found between compounds at all 5-min time increments except for

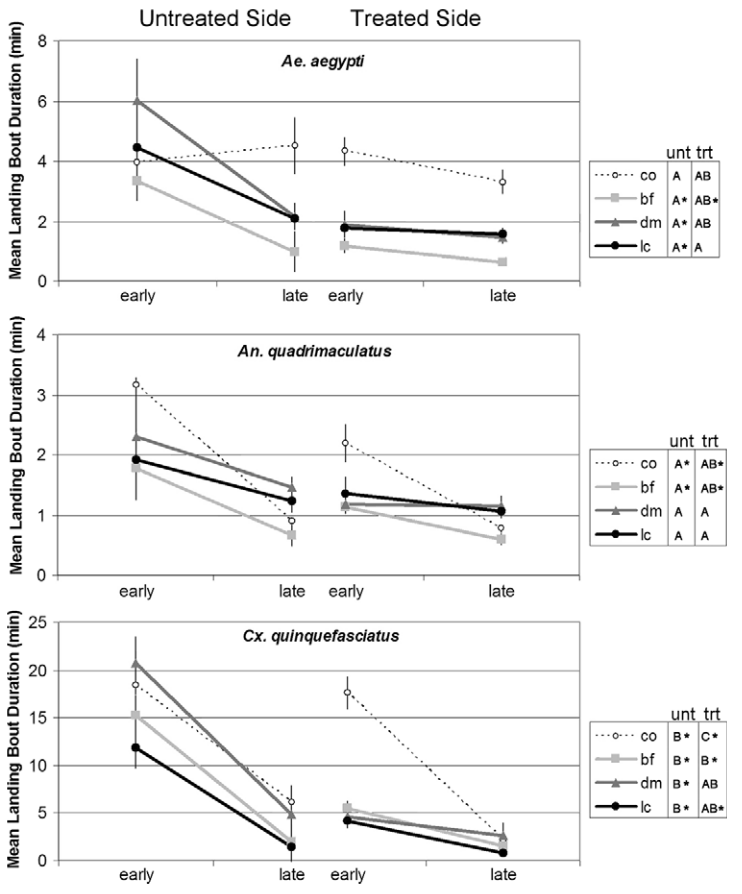


**Fig. 3.** Means of durations of landings (min) that occurred during 0–15 (early) and 15–30 min (late) for untreated (unt) and treated (trt) sides. Legends provide abbreviations for the compounds tested: co, control; bf, bifenthrin; dm, deltamethrin; and lc, lambda-cyhalothrin, and bars show SE. For each species and side, average rates of change (shown as the slope of the line) were compared, and differences between compounds are indicated in the legend, where treatments on the same side followed by the same uppercase letter are not significantly different (repeated-measures ANOVA, $P > 0.05$). Asterisks (*) indicate a significant difference between early and late average landing durations for that treatment and side (paired Student's $t$-test, $P < 0.05$). Note differences in scale between species.

0–5 min (Table 7; Fig. 4; ANOVA, $P < 0.05$). In control treatments, all three species showed a reduction in landing frequency at 5–10 min after the initial introduction into the chamber at 0–5 min. This reduction was also seen in *Ae. aegypti* and *Cx. quinquefasciatus* when exposed to any of the three pyrethroids. However, *An. quadrimaculatus* landing frequency increased immediately after introduction into the chamber when pyrethroids were present (Fig. 4).

In *An. quadrimaculatus,* landing frequency increased fastest and most dramatically in the presence of deltamethrin, with a peak at 5–10 min, followed by bifenthrin with a peak at 15–20 min and lambda-cyhalothrin with a peak between 10–20 min. *Ae. aegypti* had a gradual increase in landing frequency in the presence of bifenthrin, and *Cx. quinquefasciatus* landing frequency increased gradually in the presence of lambda-cyhalothrin and bifenthrin (Fig. 4). By the end of the experiment, *An. quadrimaculatus*

**Table 6. Average no. of mosquitoes that landed in the first 20 min and remained until the end of the exposure (to 30 min) and the average start time (min) of those landings (in parentheses; $N = 5$)**

| Species (compound) | Mean number and mean start time (min) of mosquitoes that landed in the first 20 min and remained until the end of the experiment (to 30 min) | |
|---|---|---|
| | Untreated side | Treated side |
| *Ae. aegypti* | | |
| Control | 1.4 (13.2) | 1.4 (10.1) |
| Bifenthrin | 1.6 (11.4) | 0 |
| Deltamethrin | 2.6 (5.1) | 0.2 (0.2) |
| Lambda-cyhalothrin | 2.0 (8.3) | 0 |
| *An. quadrimaculatus* | | |
| Control | 0 | 0 |
| Bifenthrin | 0.2 (15.6) | 0.2 (18.9) |
| Deltamethrin | 6.2 (11.1) | 1.6 (13.5) |
| Lambda-cyhalothrin | 0.8 (12.2) | 0 |
| *Cx. quinquefasciatus* | | |
| Control | 7.8 (3.3) | 6.8 (1.0) |
| Bifenthrin | 6.2 (3.8) | 0 |
| Deltamethrin | 5.8 (2.6) | 0 |
| Lambda-cyhalothrin | 3.0 (2.5) | 0 |

**Table 7. ANOVA table showing landing frequency differences between compounds, for each species, at different time increments**

| Species | 0–5 min | | 5–10 min | | 10–15 min | | 15–20 min | | 20–25 min | | 25–30 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| *Ae. aegypti* | 1.09 | 0.383 | 0.25 | 0.863 | 3.07 | 0.058 | 2.33 | 0.113 | 2.92 | 0.066 | 3.31 | 0.047 |
| *An. quadrimaculatus* | 2.75 | 0.077 | 25.55 | <0.001 | 11.87 | <0.001 | 5.50 | 0.009 | 2.97 | 0.063 | 2.14 | 0.135 |
| *Cx. quinquefasciatus* | 2.37 | 0.109 | 3.68 | 0.035 | 4.64 | 0.016 | 5.02 | 0.012 | 3.59 | 0.037 | 4.06 | 0.025 |

landing frequencies for deltamethrin and lambda-cyhalothrin were reduced to below that of the control.

The response was markedly different for the other two species, for which there was no difference in landing frequency between the control and deltamethrin for *Cx. quinquefasciatus* and for *Ae. aegypti* there was no difference until 25–30 min when landing frequency for deltamethrin was significantly lower than the control (Tukey, $P < 0.05$). For *Ae. aegypti*, the highest landing frequency was seen with bifenthrin, and for *Cx. quinquefasciatus*, the highest landing fre-

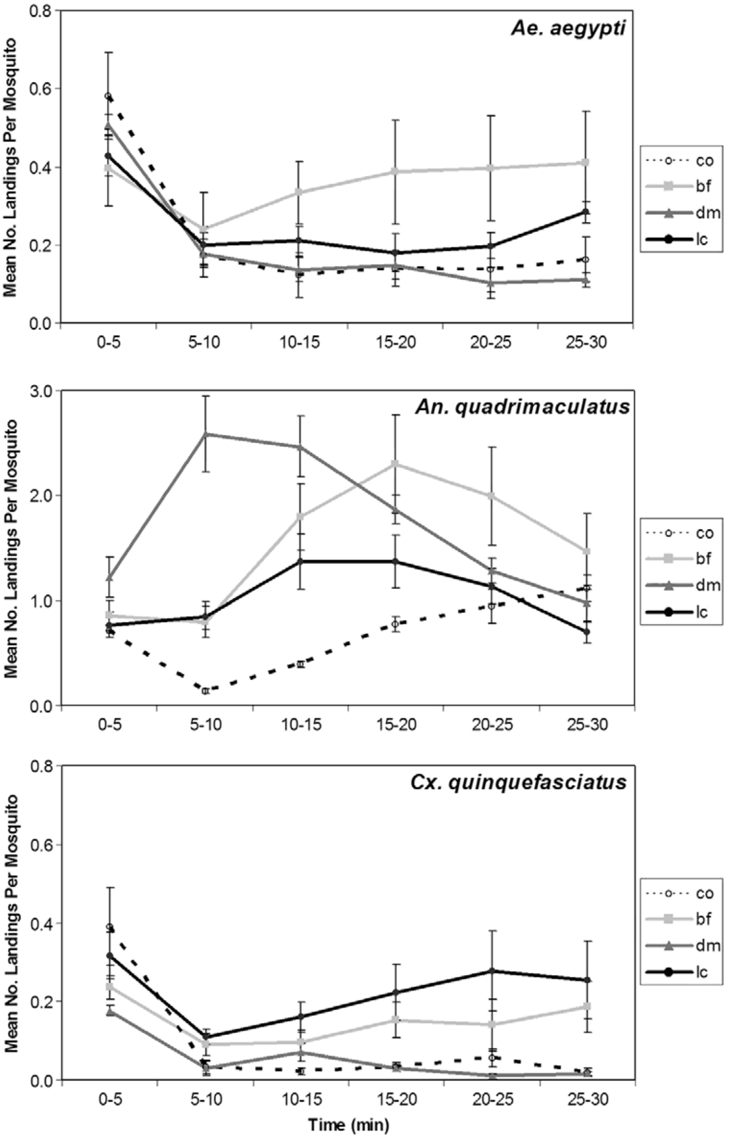


**Fig. 4.** Mean landing frequencies in 5-min time intervals for each species and compound. Legends show abbreviations for the compounds tested: co, control; bf, bifenthrin; dm, deltamethrin; and lc, lambda-cyhalothrin, and bars show SE. Note difference in scale between species.

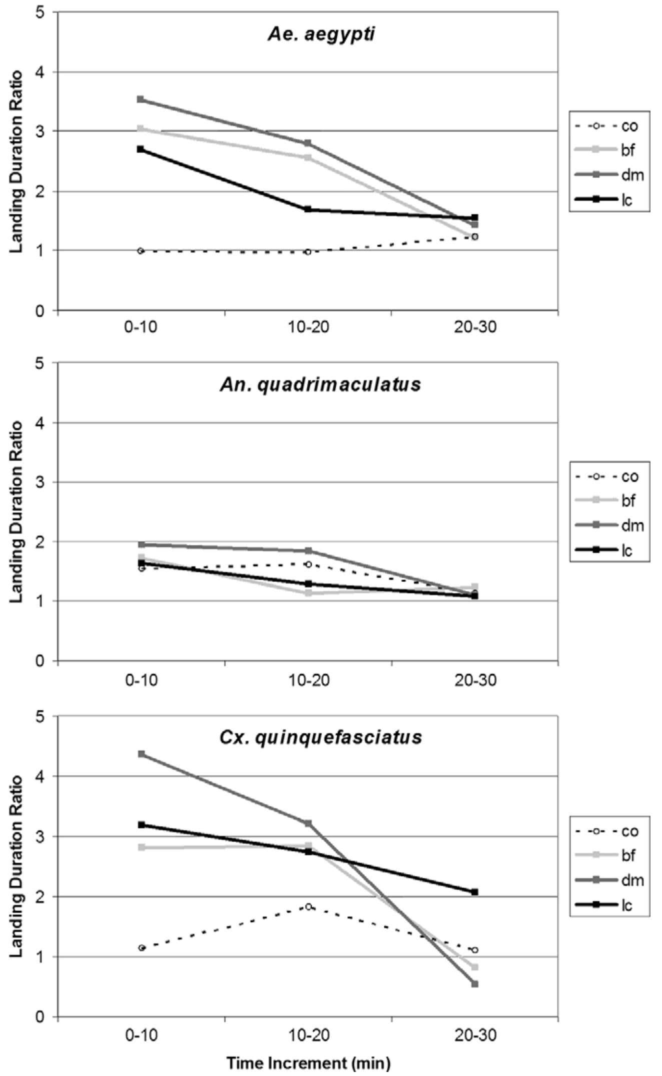


**Fig. 5.** Ratios between average landing durations on untreated and treated sides over time.

quency was seen with lambda-cyhalothrin, but both of those species had much lower landing frequencies than *An. quadrimaculatus.*

**Ratio Between Sides.** Observations were subdivided into three 10-min time increments. Within these time increments, the mean landing durations for the untreated sides were divided by those for the corresponding treated sides for each replicate. The mean ratio of landing durations are presented for every treatment. A ratio close to 1 indicates that landings on the untreated sides were of similar duration to the treated sides. A higher ratio indicates that the mean duration of landings on the untreated side was that many times longer than those on the untreated sides. Results (Fig. 5) show that, for *Ae. aegypti* and *Cx. quinquefasciatus,* initial landing durations on treated sides are shorter than on untreated sides, but this difference disappears as time progresses. This pattern did not occur for *An. quadrimaculatus,* suggesting that landings were of similar durations on both untreated and treated sides over time.

**$TK_{50}$.** Bifenthrin was the fastest at knocking down one half of the mosquitoes for all three species, with mean $TK_{50}$ ranging between 0.6 and 0.9 h, and no statistical differences between species ($P > 0.05$). Mean $TK_{50}$ were higher for both deltamethrin and lambda-cyhalothrin and similar for *Ae. aegypti* and *An. quadrimaculatus,* ranging from 2.9 to 3.7 h. However, for *Cx. quinquefasciatus,* in three cases, $TK_{50}$ was far from being reached by 10 h, but all mosquitoes were killed within 24 h. For deltamethrin, $TK_{50}$ ranged from 3.2 to >10 h, and for lambda-cyhalothrin $TK_{50}$ ranged from 7.3 to >10 h (Fig. 6).

## Discussion

**Patterns for Species.** This experiment aimed to examine whether mosquitoes would land on insecticide-

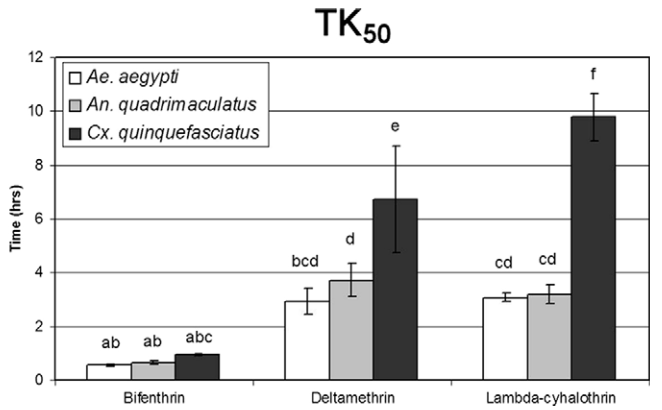


**Fig. 6.** $TK_{50}$ for three species with facultative exposure to three different pyrethroids. Bars with the same letters are not significantly different (ANOVA, $P = 0.05$).

treated surfaces when untreated surfaces were available, whether there were differences in behavior between species and compounds, and whether such differences would result in behavioral differences that ultimately affect mortality. The effects of this facultative contact with treated filter paper varied by species and compound and resulted in many interactions with few broadly applicable patterns (Table 2). However, one pattern that did hold true for all species and compounds was that landing frequency did not differ between treated and adjacent untreated surfaces.

The controls for the three species showed that, under these conditions, *An. quadrimaculatus* had the highest level of activity, followed by *Ae. aegypti* with intermediate activity, and the least active was *Cx. quinquefasciatus.* Differences between controls suggest that there may be innate differences between the three species with respect to activity levels, time of day, or preferences for landing on surfaces of various textures or orientation. For *Ae. aegypti* and *Cx. quinquefasciatus,* when pyrethroids were present, most changes in behavior occurred on treated surfaces rather than adjacent untreated surfaces. However, for *An. quadrimaculatus,* changes in behavior could be seen on both surfaces (Table 2).

**Landing Frequency.** Although the mean number of landings per mosquito differed from one treatment to another, at no time in the experiment did landing frequency differ between the untreated side and the treated side, suggesting that treated surfaces were neither repellent nor attractive compared with untreated sides.

Landing frequency over time differed depending on the presence and type of insecticide, as well as species, but not side (Fig. 4). After the initial 5 min when mosquitoes were aspirated into the chamber, *Cx. quinquefasciatus* landing frequency became the lowest of the three species, and it changed the least as time progressed. Landing frequency was also initially low for *Ae. aegypti,* but with exposure to bifenthrin, it increased gradually over time, suggesting a slight and gradual excitatory effect. For *An. quadrimaculatus,* rather than an initial decrease in landing frequency after introduction into the chamber as in the control, there was a sharp increase with exposure to deltamethrin, indicating a relatively strong and immediate excitatory effect with that species and compound. With bifenthrin and lambda-cyhalothrin, there were also strong increases in landing frequency that occurred several minutes later. By the end of the experiment, landing frequency returned to a level similar to the control.

**Landing Durations.** In the first half of the experiment, landing durations on treated surfaces were shorter compared with untreated surfaces or controls. On untreated surfaces, the likelihood that any mosquito had acquired a dose of insecticide from the treated side increased with time, and there was a commensurate reduction in landing durations on untreated surfaces as time progressed.

For deltamethrin and *An. quadrimaculatus,* there were some unusual results. Females landed on the untreated side and remained until the end of the trial more often than with other treatments. There was also a slight increase from zero in the number resting on the treated side until the end. This suggests that deltamethrin, in addition to the excitatory effect with regard to landing frequency, may have had a slightly depressive effect with this species, which was not seen with other compounds. For *An. quadrimaculatus* with deltamethrin, the depressive effect coupled with the greater landing frequency was also reflected in the number of *An. quadrimaculatus* females resting on both sides of deltamethrin in the snapshots over time (Table 5), as well as total contact time (Table 3a, b), and total resting time for both sides summed (Table 2).

When averaged over 30 min, there was a general trend that landing duration and total contact time were greater on untreated sides compared with the treated surfaces for all species and compounds. For each species, these differences were statistically significant for different compounds: deltamethrin for *Cx. quinquefasciatus,* bifenthrin for *An. quadrimaculatus,* and both lambda-cyhalothrin and deltamethrin for *Ae. aegypti.* However, data averaged over 30 min should not be interpreted alone because, as we have shown, over time, behaviors changed dramatically and in different ways depending on the species and compound.

When contact was made with a pyrethroid-treated surface, whether in the first or last 15 min, the landings were shorter than those on the untreated surface in the first 15 min. However, the landing durations on the untreated sides in the last 15 min were more similar to those on the treated sides. This is likely because a modification in landing duration occurred only after mosquitoes had the opportunity to come into contact and acquire a dose of pyrethroid, at which time their landings became shorter than those that did not have prior contact.

**Summary and Sequence of Behavioral Responses.** Mosquitoes landed on both surfaces with equal frequency, but landings on treated sides were shorter than landings on untreated sides. Therefore, mosquitoes spent less time on treated surfaces. Mosquitoes with a sublethal dose after landing on the treated side had shorter and more frequent landings on any surface.

Because as time progressed the chance of contact with a treated side increased, only mosquitoes on untreated surfaces early in the experiment had longer landing durations (Figs. 3 and 5).

Shorter landing durations coupled with overall low landing frequencies are probably responsible for fewer *Ae. aegypti* and *Cx. quinquefasciatus* females on surfaces in snapshots over time, and the higher landing frequency in *An. quadrimaculatus* probably accounts for their greater numbers seen in snapshots (Table 5). Most snapshots did not show a change in total number of mosquitoes resting on both sides combined, except with deltamethrin, where there were clearly more *An. quadrimaculatus* and fewer *Ae. aegypti* than on their respective controls. Other compounds showed reductions in numbers mainly on treated sides. Lambda-cyhalothrin did not seem to affect the number of *An. quadrimaculatus* on either side.

If the 60 mosquitoes in the cage randomly distributed themselves among the six walls, we would expect to see on average 10 mosquitoes shared between the two adjacent filter papers. In controls, we see totals close to this with *Ae. aegypti* and higher than expected with *An. quadrimaculatus* and *Cx. quinquefasciatus.* In pesticide treatments, we see shifts from one filter paper to the other, often not affecting the overall total on the two papers. However, in certain instances, we see an increase or decrease from expected (Table 5).

Because *Cx. quinquefasciatus* landed infrequently, and landing durations were long, reductions in numbers on treated surfaces stood out, whereas numbers on untreated surfaces did not change much. This pattern was weaker with *Ae. aegypti,* and nonexistent with *An. quadrimaculatus*, which had a high landing frequency and relatively short landings.

**Behavioral Resistance.** Although contact with insecticide-treated filter paper was facultative, lethal doses were acquired by all three species. Bifenthrin had the fastest $TK_{50}$ for all species with facultative exposure. The $TK_{50}$ for *Cx. quinquefasciatus* was slower than the other species, especially with facultative exposure to lambda-cyhalothrin and deltamethrin. This is consistent with other studies that found lower mortality for *Cx. quinquefasciatus* compared with species of *Aedes* or *Anopheles* when exposed to certain insecticides in laboratory and field studies (Ansari et al. 1998, Ham et al. 1999). Although mechanisms of physiological resistance certainly exist (Chandre et al. 1998, 1999a, b), mechanisms of behavioral resistance that reduce exposure to insecticides could also be involved (Hostetler and Brenner 1994). Perhaps behavioral patterns such as those seen in *Cx. quinquefasciatus* in Tables 5 and 6 can be viewed as a source of behavioral resistance, also keeping in mind that knockdown did not begin until well after 30 min.

**Terminology with Regard to Insect Behavior and Responses to Insecticides.** Kennedy (1947), who ran similar experiments on *Ae. aegypti* and *An. atroparvus* Van Thiel exposed to filter papers treated with DDT, described a set of behaviors he observed in mosquitoes experiencing the toxic effects of DDT. These behaviors were "excitation, ataxia, convulsions, general paralysis (knock-down) and, ultimately, death." Although the excitation as a behavior was discussed, it was not defined. One logical definition of the term excitation involves an increase in any measured behavior. Locomotive excitation would presumably involve an increase in time or intensity spent walking or flying and could be so specific as to refer to an increase in speed or turning (kinetic movement), or other activities related to locomotion. In fact, the result of such locomotive excitation would be kinetic (unoriented) movement away from the starting location (Fraenkel and Gunn 1940).

The origin of the term excito-repellency seems to be a paper by Rachou et al. (1963) entitled "Experiences with the excito-repellency test box—model OPS," in which they described a prototype of a device they designed to study "the combined effect of irritation and repellency of free-flying mosquitoes, even if it did not measure the two phenomena separately." The term was adopted the following year by Busvine (1964) to describe "refractory behavior in the presence of insecticides." Since its inception, this term has generally been used to describe a behavioral endpoint, the overall movement away from the area, as a result of excitation caused by insecticides (see also White 2007). However, the term repellent was defined by Dethier et al. (1960) as "a chemical which causes insects to make oriented movements away from its source." Barton Browne (1977) defined a repellent as a chemical vapor that "causes an insect to behave in ways which result in its movement away from the source of the material." Roberts et al. (2000) added that a repellent requires that there be no tarsal contact and that chemicals causing "oriented movement of avoidance after tarsal contact" were termed "irritants." Although the name excito-repellency suggests that there are two behaviors taking place as a result of exposure to certain compounds, excitation and repellency, the latter may not technically be taking place because it is neither oriented nor working as a vapor. In fact, landing was equally frequent on treated and untreated sides, and our data suggest that, in most cases, excitation was a result of acquiring a dose of insecticide through contact with the treated surface. It seems that the excitation documented here as a result of exposure to pyrethroids aligns with the definition of Dethier et al. (1960) of a "locomotor stimulant," which is described as "a chemical which causes, by a kinetic mechanism, insects to disperse from a region more rapidly than if the area did not contain the chemical. The effect may be to increase the speed of locomotion, to cause the insects to carry out avoiding reactions, or to decrease the rate of turning (Fraenkel and Gunn 1940)."

Excitation can be an increase in any type of behavior, such as walking, flying, or even grooming. It can also apply to the intensity of a movement, such as an increase in speed (orthokinesis) or frequency of turning (klinokinesis) (Kennedy 1977). Increasing speed and/or decreasing turning, when unoriented, can increase the likelihood of movement away from the original position. When pursuing an understanding of the mechanisms behind the apparent repellency, special attention should be applied to the type of excitation involved. Because the term excito-repellency describes this end result rather than the behavioral process and is suggestive of a mechanism that may not be correct, we suggest that the more appropriate term is the one that describes the modality, in this case, "excitation" or "locomotory stimulant." An important aspect of behavior that was not examined by this experiment was what mosquitoes did in flight, and whether the excitation caused by exposure to insecticides elicited a change in turning or velocity while flying. It is likely that future studies examining the behavioral effects of sublethal exposure of mosquitoes to pyrethroids will show an increase in speed and possibly a change in turning, which may explain observations of kinetic movement away from the chemical.

## Acknowledgments

Our sincere gratitude is extended to C. Dickerson for critical review of this manuscript and constructive discussions regarding the statistical analysis, S. Su for reviewing this manuscript, and C. Geden for helpful suggestions. This research was made possible by funding from the Deployed War-fighter Protection Program.

## References Cited

**Ansari, M. A., N. Kapoor, and V. P. Sharma. 1998.** Relative efficacy of synthetic pyrethroid-impregnated fabrics against mosquitoes under laboratory conditions. J. Am. Mosq. Control Assoc. 14: 406–409.

**Barton Browne, L. 1977.** Host-related responses and their suppression: some behavioral considerations, pp. 117–127. *In* H. H. Shorey and J.J.J. McKelvey (eds.), Chemical control of insect behavior: theory and application. Wiley, New York.

**Busvine, J. R. 1964.** The significance of DDT-irritability tests on mosquitoes. Bull. W.H.O. 31: 645–656.

**Chandre, F., F. Darriet, M. Darder, A. Cuany, J.M.C. Doannio, N. Pasteur, and P. Guillet. 1998.** Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. Med. Vet. Entomol. 12: 359–366.

**Chandre, F., F. Darrier, L. Manga, M. Akogbeto, O. Faye, J. Mouchet, and P. Guillet. 1999a.** Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. Bull. W.H.O. 77: 230–234.

**Chandre, F., F. Darriet, S. Manguin, C. Brengues, P. Carnevale, and P. Guillet. 1999b.** Pyrethroid cross resistance spectrum among populations of Anopheles gambiae s.s. from Cote d'Ivoire. J. Am. Mosq. Control Assoc. 15: 53–59.

**Coleman, M., and J. Hemingway. 2007.** Insecticide resistance monitoring and evaluation in disease transmitting mosquitoes. J. Pestic. Sci. 32: 69–76.

**Conover, W. J. 1980.** Practical nonparametric statistics. Wiley, New York.

**Corbel, V., F. Chandre, C. Brengues, M. Akogbeto, F. Lardeux, J. M. Hougard, and P. Guillet. 2004.** Dosage-dependent effects of permethrin-treated nets on the behaviour of *Anopheles gambiae* and the selection of pyrethroid resistance. Malaria J. 3: 22.

**Dethier, V. G., L. B. Browne, and C. N. Smith. 1960.** The designation of chemicals in terms of the responses they elicit from insects. J. Econ. Entomol. 53: 134–136.

**Eamsila, C., S. P. Frances, and D. Strickman. 1994.** Evaluation of permethrin-treated military uniforms for personal protection against malaria in northeastern Thailand. J. Am. Mosq. Control Assoc. 10: 515–521.

**Evans, R. G. 1993.** Laboratory evaluation of the irritancy of bendiocarb, lambda-cyhalothrin and DDT to *Anopheles gambiae.* J. Am. Mosq. Control Assoc. 9: 285–293.

**Fraenkel, G. S., and D. L. Gunn. 1940.** The orientation of animals. The Clarendon Press, Oxford, United Kingdom.

**Gerberg, E. J., D. R. Barnard, and R. A. Ward. 1994.** Manual for mosquito rearing and experimental techniques. American Mosquito Control Association, Lake Charles, LA.

**Ham, C. M., M. V. Meisch, and C. L. Meek. 1999.** Efficacy of Dibrom(R), Trumpet(R), and Scourge(R) against four mosquito species in Louisiana. J. Am. Mosq. Control Assoc. 15: 433–436.

**Hostetler, M. E., and R. J. Brenner. 1994.** Behavioral and physiological resistance to insecticides in the German cockroach (Dictyoptera: Blattellidae): an experimental reevaluation. J. Econ. Entomol. 87: 885–893.

**Hougard, J. M., S. Duchon, F. Darriet, M. Zaim, C. Rogier, and P. Guillet. 2003.** Comparative performances, under laboratory conditions, of seven pyrethroid insecticides used for impregnation of mosquito nets. Bull. W.H.O. 81: 324–333.

**Kapoor, N., and M. A. Ansari. 2003.** Laboratory evaluation of synthetic pyrethroid-treated cotton fabric against mosquitoes and other domestic pests. J. Trop. Med. Parasitol. 26: 20–25.

**Kennedy, J. S. 1947.** The excitant and repellent effects on mosquitoes of sub-lethal contacts with DDT. Bull. Entomol. Res. 37: 593–607.

**Kennedy, J. S. 1977.** Olfactory responses to distant plants and other odor sources, pp. 67–92. *In* H. H. Shorey and J. J. Mckelvey, Jr. (eds.), Chemical control of insect behavior: theory and application. Wiley, London, United Kingdom.

**Kongmee, M., A. Prabaripai, P. Akratanakul, M. J. Bangs, and T. Chareonviriyaphap. 2004.** Behavioral responses of *Aedes aegypti* (Diptera: Culicidae) exposed to deltamethrin and possible implications for disease control. J. Med. Entomol. 41: 1055–1063.

**Miller, J. E., and G. Gibson. 1994.** Behavioral response of host-seeking mosquitoes (Diptera: Culicidae) to insecticide-impregnated bed netting: a new approach to insecticide bioassays. J. Med. Entomol. 31: 114–122.

**Montgomery, D. C. 2005.** Design and analysis of experiments. Wiley, Hoboken, NJ.

**Pates, H., and C. Curtis. 2005.** Mosquito behavior and vector control. Annu. Rev. Entomol. 50: 53–70.

**Perich, M. J., M. A. Tidwell, S. E. Dobson, M. R. Sardelis, A. Zaglul, and D. C. Williams. 1993.** Barrier spraying to control the malaria vector *Anopheles albimanus:* laboratory and field evaluation in the Dominican Republic. Med. Vet. Entomol. 7: 363–368.

**Posey, K. H., and C. E. Schreck. 1981.** An airflow apparatus for selecting female mosquitoes for use in repellent and attraction studies. Mosq. News 41: 566–568.

**Rachou, R. G., M. Moura-Lima, J. P. Duret, and J. A. Kerr. 1963.** Experiences with the excito-repellency test box—model OPS, pp. 442–447. *In* Proceedings of the 50th Annual Meeting of the New Jersey Mosquito Extermination Association and the 19th Annual Meeting of the American Mosquito Control Association, 12 March 1963, Atlantic City, NJ.

**Roberts, D. R., and W. D. Alecrim. 1991.** Behavioral response of *Anopheles darlingi* to DDT-sprayed house walls in Amazonia. Bull. PAHO 25: 210–217.

**Roberts, D. R., T. Chareonviriyaphap, H. H. Harlan, and P. Hshieh. 1997.** Methods of testing and analyzing excito-repellency responses of malaria vectors to insecticides. J. Am. Mosq. Control Assoc. 13: 13–17.

**Roberts, D. R., W. D. Alecrim, P. Hshieh, J. P. Grieco, M. Bangs, R. G. Andre, and T. Chareonviriphap. 2000.** A probability model of vector behavior: effects of DDT repellency, irritancy, and toxicity in malaria control. J. Vector Ecol. 25: 48–61.

**SAS Institute. 2003.** SAS computer program, version 9.1. SAS Institute, Cary, NC.

**Sokal, R. R., and J. F. Rohlf. 1995.** Biometry. W. H. Freeman and Company, New York.

**Takken, W. 2002.** Do insecticide-treated bednets have an effect on malaria vectors? Trop. Med. Int. Health 7: 1022–1030.

**Vijverberg, H.P.M., J. M. van der Zalm, and J. van den Bercken. 1982.** Similar mode of action of pyrethroids and DDT on sodium channel gating in myelinated nerves. Nature (Lond.) 295: 601–603.

**White, G. B. 2007.** Terminology of insect repellents, pp. 31–44. *In* M. Debboun, S. Frances, and D. Strickman (eds.), Insect repellents: principles, methods and uses. CRC, London, United Kingdom.

*Received 25 February 2008; accepted 8 November 2008.*